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PERSPECTIVE

Gene expression profiling and cardiac allograft rejection monitoring: Is IMAGE just a mirage?

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The search for an effective non-invasive monitoring technique for cardiac allograft rejection eluded us until the discovery and validation of a commercially available gene-based peripheral blood bio-signature signal. The Invasive Monitoring Attenuation through Gene Expression (IMAGE) trial tested the hypothesis of cardiac biopsy minimization using this gene-based panel in stable, low-risk survivors, late after cardiac transplantation and demonstrated non-inferiority of this strategy. We present a clinician's critical perspective on this important effort and outline the key caveats and highlights for the potential way forward in using these results. Furthermore, we contend that it may not be necessary to replace an invasive cardiac biopsy strategy with anything other than better standardized clinical and functional allograft vigilance in low-risk survivors.
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The search for an effective non-invasive monitoring technique for cardiac allograft rejection eluded us until the discovery and validation of a commercially available gene-based peripheral blood transcriptomic signal. This particular bio-signature was painstakingly developed in a multicenter investigation with the primary intent of identifying immunologic quiescence.[1] The informative genes included in this gene expression panel represent a number of biologic pathways, including T-cell activation (*PDCD1*), T-cell migration (*ITGA4*), and mobilization of hematopoietic precursors (*WDR40A* and microRNA gene family *cMIR*), and steroid-responsive genes such as *IL1R2*, the decoy receptor for interleukin-2.

Early investigations also supported the development of this panel as a clinical tool to predict future risk of immunologic activity in the cardiac allograft.[2,3] In this regard, the gene-based panel provides a distinct advantage over a cardiac biopsy specimen because there is no known prognostic value to a negative biopsy specimen in predicting the outcome from subsequent testing.

Although clinical use ensued for this non-invasive marker test, clinician comfort and payor endorsement were difficult to achieve due to a lack of an effective, well-constructed clinical trial that proposed a meaningful level of evidence to guide decision making. The Invasive Monitoring Attenuation through Gene Expression (IMAGE) trial, presented at the Thirtieth Anniversary Meeting and Scientific Sessions of the International Society of Heart and Lung Transplantation (April 21 to April 24 2010, Chicago, Il) and published concurrently,[4] effectively sought to close the evidence gap and ensure the appropriate clinical placement of this non-invasive gene-based bio-signature strategy. Did the IMAGE trial achieve this goal, or are we still left in considerable doubt? To discuss this, we need to analyze the trial details in a careful manner.

The IMAGE trial sought to enroll a population of adult cardiac transplant survivors late after transplantation, at 12 months to 5 years (later modified to include 6 to 12 months after transplantation as well), who were at low risk of cardiac events. The study excluded those with a significant history of rejection, cardiac allograft vasculopathy, or allograft dysfunction.

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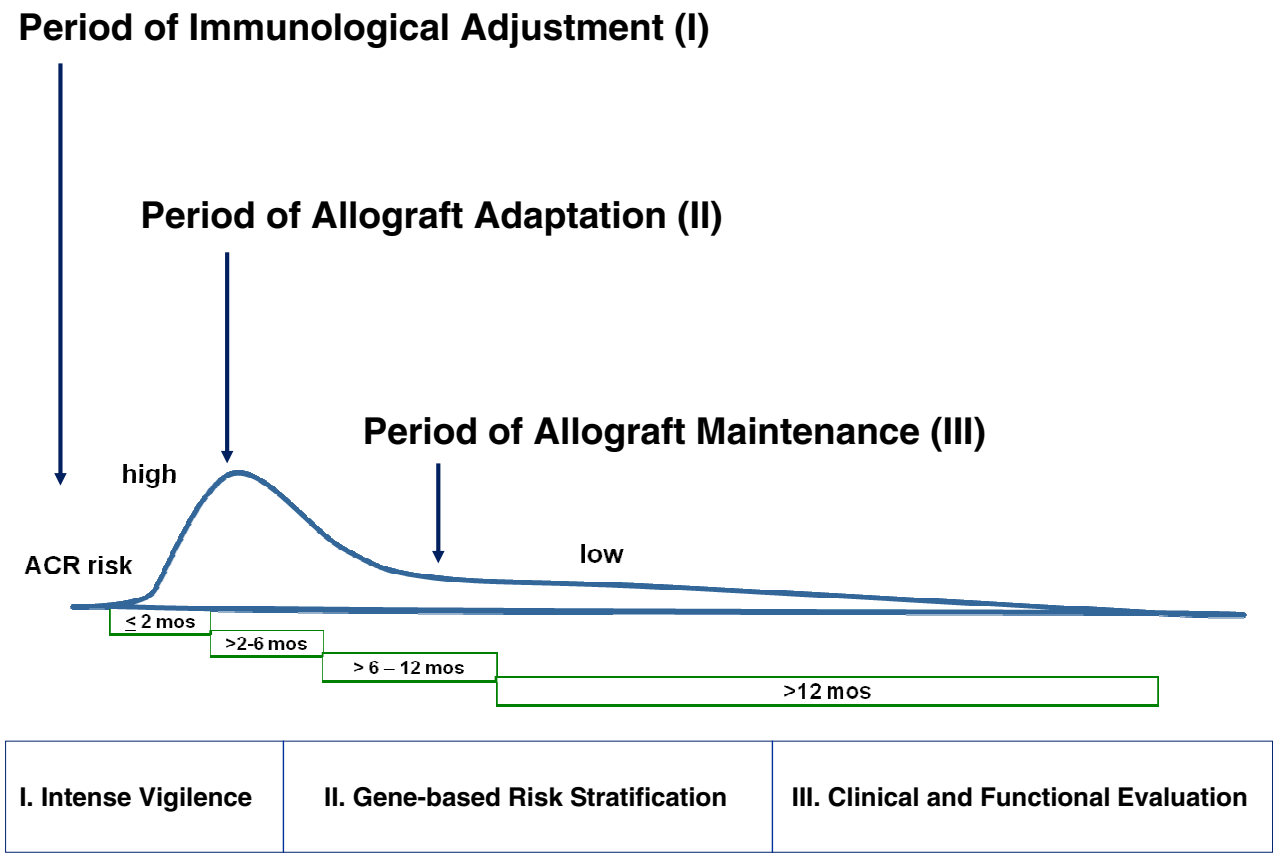

Figure 1 Time-dependent risk of cardiac rejection requires dynamic surveillance strategies: The 3 distinct periods of immunologic adjustment (early), allograft adaptation, and then the stable phase of allograft maintenance require distinct vigilance strategies. Early on, a very concerted and aggressive strategy is required while adjustment of immunosuppression is underway. In Months 2 to 6, as allograft adaptation ensues, risk stratification for intense vs less intense strategies may be needed, perhaps guided by the predictive ability of gene-expression testing. In the maintenance phase, a clinical and functional evaluation structured approach may be adequate.

Patients were then randomly allocated in a non-blinded design to an initial invasive endomyocardial biopsy-guided strategy ($n = 305$) or gene expression profiling ($n = 297$). Crossovers to the invasive strategy were allowed for abnormal clinical or echocardiography findings suggesting allograft dysfunction. If the gene expression signature resulted in a score of 34 or greater (on a scale of 0–40; threshold denotes a "higher risk for lack of quiescence"), an endomyocardial biopsy strategy was immediately used.

The groups were monitored for 2 years for hard clinical events, including allograft loss, rejection with hemodynamic compromise, or non-specific allograft failure. The aggregate results suggest that an initial non-invasive strategy using a gene-based biomarker-monitoring test is no different in 2-year outcomes than an initial protocol-driven invasive cardiac biopsy strategy, with cumulative event rates of 14.5% and 15.3%, respectively, and a hazard ratio of 1.04 (95% confidence interval [CI], 0.67–1.68). These surface findings deserve a deeper and more refined analysis.

Are protocol cardiac biopsies a "validated" strategy late after transplantation?

The frequency of performing cardiac biopsies, especially late after stability has ensued in cardiac transplantation, has never been scientifically authenticated as sound but is anecdotally assumed to improve outcomes. This remains an unproven standard in our clinical armamentarium. The practice evolved from the need to perform intense surveillance during early immunologic adaptation between 0 and 6 months after transplantation and has persisted even into the period of maintenance (>6 months), when the frequency of cardiac rejection detection falls considerably and is highly variable and negligible with time elapsed (>2 years; Figure 1).

Surveillance biopsies were initially introduced in the pre-cyclosporine era, when the frequency and clinical manifestations of acute rejection were vastly different from current practice. Not only is the ongoing utility of routine cardiac biopsy questionable in low-risk-profile individuals, it is also unclear whether the clinical implications of a finding of histologic rejection on a late biopsy specimen are similarly ominous to that of a specimen-proven rejection episode within the first 6 months of engraftment.[5] Indeed, investigations have suggested a more benign course for late rejections by demonstrating a high rate of spontaneous recovery, even when left untreated.[6]

The initial assumptions of the IMAGE trial investigators for event rate frequency were not met, which likely prompted them to alter their protocol to include earlier post-transplantation time frames. Originally, the IMAGE

trial only enrolled patients after the first year of transplantation, and this was modified to >6 months midway into the trial.[7] Owing to the uncertain significance of specimens from surveillance cardiac biopsies late after transplantation and the recognition that the yield is very low, most centers have evolved highly variable protocols for monitoring rejection late after transplantation, and most believe that the usefulness of this invasive strategy after the first year is doubtful. Thus, the "control" strategy tested in the IMAGE study rests on a poorly validated, clinically divergent, and inconsistent algorithm.

Is there clear biologic plausibility of the trial end points chosen for evaluation with the strategy of surveillance?

The premise of obtaining cardiac biopsy specimens is to detect early histologic changes that can be preempted therapeutically to prevent progression to advanced stages of allograft rejection that result in functional compromise. Similarly, the gene expression-profiling test was initially designed to pick up signals for acute cellular rejection or quiescence in cohorts of patients without cardiac allograft dysfunction. Although the IMAGE trial end points are clinically very meaningful—rejection with hemodynamic compromise, non-specific allograft failure, or death—these events were mostly not predicted by the gene-based test and were often first events that were not predicted by biopsy specimen findings. One would not expect the cardiac biopsy specimen or the gene-based profiling test to pick up current or future non-rejection-related cardiac allograft failure, an end point that populated 33% of the eventual events in the IMAGE trial.

Antibody-mediated rejection is also not predicted by previous cellular rejection on histology, and the diagnosis of this particular entity remains difficult.[8] Standards to diagnose antibody-mediated rejection have only been recently developed and as yet remain less widely adopted, with marked variability in interpretation.[9,10] Similarly, in the absence of knowing how robustly the 2 strategies predicted the subsequent events in groups studied or averted potential such events, there must be considerable doubt on the usefulness of either approach for late monitoring.

Are the trial results broadly applicable and are statistically designed non-inferiority margins clinically valid?

A non-inferiority trial tests the assumption that one clinical strategy is no worse than another by a pre-specified delta difference and, therefore, is not a true test of "equivalence." The boundaries of non-inferiority must not be developed arbitrarily to suit the trial size or expected event rates to create trial duration assumptions. Instead, these boundaries must be robustly developed based on clinically valid assumptions of the true tolerance of differences of the strategy being tested with the control strategy, within reason.[11] Further, for a strategy to be statistically non-inferior, the upper 95% CI of the strategy being tested as non-inferior must be well below the pre-specified delta. In the context of the IMAGE trial, the investigators chose a twofold higher boundary for non-inferiority (2.054) in favor of the gene expression profiling test and the upper 95% CI value fell below this level (1.68). This means that the gene profiling test strategy could be as much as 68% worse than the biopsy strategy and yet be interpreted as non-inferior. Certainly, one critical assumption is that the invasive cardiac biopsy strategy is proven to be better than clinical assessment alone, but that too remains unknown.

The inherent discomfort in such analyses also lies in the translation to the real world. We know that strategies or therapeutic maneuvers are rarely used in their mandated parameters and often used "off label." This will likely be the case with gene expression testing, and one must be careful to ensure that an isolated gene expression–based profiling strategy, in some circumstances, should either not be done or should be coupled with greater ongoing vigilance by the clinician.

The generalization to broad populations is also in question, because only 1 in 5 patients who were screened (n = 2946) were eventually randomized (n = 602) into IMAGE; thus, one must not assume that the IMAGE trial is applicable for all post-transplant late survivors. Furthermore, one must be very careful in adopting this approach in the 6-month to 1-year post-transplant group because the entry of this cohort was a mid-protocol modification and represents <15% of the patients enrolled. In subsequent analyses, it will be important to know in what time periods the eventual events occurred and the frequency of potentially averted events.

Has the role of clinical assessment and echocardiography been underemphasized?

One of the lynchpins of any rejection surveillance protocol is a well-conducted clinical evaluation for de novo symptoms and sub-clinical cardiac allograft dysfunction. The notion that echocardiography is useful in only identifying allograft failure must be tempered by the fact that one can pick up early signs of allograft compromise by detection of myocardial thickening (potentially representing edema), ventricular dyssynchrony, diastolic dysfunction, or valvular regurgitation.[12] Although less accurate, estimated pressures from Doppler flow analysis allow hemodynamic calculations. In fact, it is more plausible that use of an echocardiography-based clinical strategy would be more directly linked to picking up all hierarchal components of the IMAGE trial end points (whether rejection-linked or not) and thus be a more effective preemptive surveillance strategy for allograft dysfunction due to causes other than cellular rejection. In essence, an evolved and well-structured echocardiography and clinical assessment alone may clinically trump the usefulness of invasive or gene-based biomarker strategies, and the question must be asked if we can now be comfortable in pursuing that line of investigation.

All in all, the IMAGE trial is an important step forward and will help us in systematically understanding the utility or lack of usefulness of poorly validated clinical algorithms. Just as renal and hepatic transplantation programs have divested themselves of protocol-driven surveillance and evolved into clinically mandated rejection detection algorithms, the IMAGE trial may eventually point us in the direction of strategies guided by clinical vigilance guided and result in greater comfort in divesting ourselves from the psychologic need to perform low-yield and expensive tests in the maintenance of stable post-transplant survivors who are at low risk of events. It will be important to analyze the crossover rates, protocol deviations, and histologic findings of various strategies and also the influence of therapeutic interventions on outcomes in the IMAGE trial. Even more important will be the correlative analysis of surveillance events with eventual hard events, longitudinal gene expression findings, and the findings from the echocardiography core laboratory analysis.

Where should we go from here? The low event rates in the primary rejection domain must give clinicians great comfort in critically reviewing their surveillance strategies, especially in stable low-risk patients >1 year after transplantation. We believe that the best concentration of future investigation in the area of gene expression profiling is in early prediction of future rejection close to the engraftment time point, and this notion needs to be appropriately tested and corroborated (Figure 1).

In summary, we contend that the invasive strategy likely suffers from potentially high false-negative rates as well as false-positives, whereas the gene-based testing gives us more false-positives and even false-negative rates when the end points are those beyond the assessment of histologic quiescence. It is entirely possible that neither strategy is useful in improving clinical outcomes in low-risk survivors late after cardiac transplantation. Clinicians must remain very vigilant in not extrapolating these findings to survivors at higher risk, such as those with recurrent or persistent rejection, those with destabilization of immunosuppressant regimens, or those with high-risk demographic profiles, such as multiparous women, donor-specific antibody producers, blacks, and retransplantation.

Although we understand and appreciate that change in clinical practice must often take small steps, we believe that these findings provide us with enough equipoise to test a strategy of doing away with excessive and protocol biopsies in well-selected low-risk heart transplantation survivors after 1 year, and furthermore contend that it may not be necessary to replace that strategy with anything other than better standardized clinical and functional allograft vigilance.

Disclosure statement

Dr Mehra has formerly received research grants or consulting fees from XDX Inc (>12 months). Dr Parameshwar participated in Cardiac Allograft Rejection Gene Expression Observational (CARGO) II trial (>12 months). These views represent the sole views of the authors and not those of the collective International Society of Heart and Lung Transplantation or the *Journal of Heart and Lung Transplantation* leadership.

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